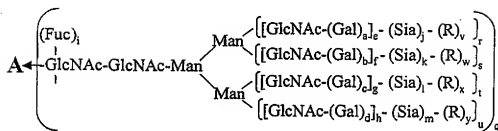
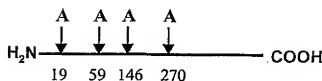


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a-d, i, q-u (independently selected) = 0 or 1.

e-h (independently selected) = 0 to 6.

j-m (independently selected) = 0 to 100.

v-y = 0; R = polymer.

FIG. 39A

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CHO, BHK, 293 cells, Vero expressed Cerezyme  
a-d, i-m, q-u (independently selected) = 0 or 1;  
e-h = 1; v-y = 0.

- ↓
1. Sialidase
  2. CMP-SA-PEG (16 mol eq),  
ST3Gal3

a-d, i-m, q-u (independently selected) = 0 or 1;  
e-h = 1; v-y (independently selected) = 1,  
when j-m (independently selected) is 1;  
R = PEG.

FIG. 39B

CHO, BHK, 293 cells, Vero expressed Cerezyme.  
a-d, i-m, q-u (independently selected) = 0 or 1;  
e-h = 1; v-y = 0.

- ↓
1. Sialidase
  2. CMP-SA-M-6-P (1.2 mol eq),  
ST3Gal3
  3. CMP-SA (16 mol eq), ST3Gal3

a-d, i-m, q-u (independently selected) = 0 or 1;  
e-h = 1; v-y (independently selected) = 0 or 1;  
R = mannose-6-phosphate

FIG. 39C

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CHO, BHK, 293 cells, Vero expressed Cerezyme.  
a-d, i-m, q-u (independently selected) = 0 or 1;  
e-h = 1; v-y = 0.

- ↓
1. Sialidase
  2. CMP-SA-PEG (16 mol eq),  
ST3Gal3
  3. CMP-SA, ST3Gal3

a-d, i-m, q-u (independently selected) = 0 or 1;  
e-h = 1; v-y (independently selected) = 0 or 1;  
R = Mannose-6-phosphate

FIG. 39D

CHO, BHK, 293 cells, Vero expressed Cerezyme.  
a-d, i-m, q-u (independently selected) = 0 or 1;  
e-h = 1; v-y = 0.

- ↓
1. CMP-SA-levulinate, ST3Gal3,  
buffer, salt
  2.  $H_4N_2$ -spacer-M-6-P or clustered M-6-P

a-d, i-m, q-u (independently selected) = 0 or 1;  
e-h = 1; v-y (independently selected) = 0 or 1;  
R = M-6-P or clustered M-6-P

FIG. 39E

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CHO, BHK, 293 cells, Vero expressed Cerezyme.  
a-d, i-m, q-u (independently selected) = 0 or 1;  
e-h = 1; v-y = 0.

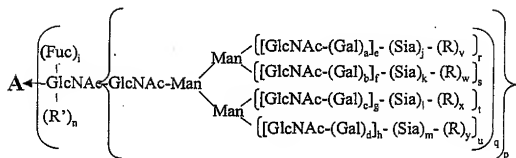


1. CMP-SA,  $\alpha$ 2,8-ST

a-d, i, q-u (independently selected) = 0 or 1;  
e-h = 1; j-m (independently selected) = 0-20;  
v-y (independently selected) = 0.

FIG. 39F

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a-d, i, n, p-u (independently selected) = 0 or 1.

e-h (independently selected) = 0 to 6.

j-m (independently selected) = 0 to 100.

v-y = 0;

R = modifying group, mannose, oligo-mannose;

R' = H, glycosyl residue, modifying group,  
glycoconjugate.

FIG. 39G

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Insect cell expressed Cerezyme.

a-d, f, h, j-m, s, u, v-y = 0;

e, g, i, q, r, t (independently selected) = 0 or 1.



1. GNT's 1,2,4,5, UDP-GlcNAc

2. Galactosyltransferase, UDP-Gal-PEG

a-i, q-u (independently selected) = 0 or 1;

j-m = 0;

v-y (independently selected) = 1,

when e-h (independently selected) is 1;

R = PEG.

FIG. 39H

Yeast-expressed Cerezyme.

a-m = 0; q-y (independently selected) = 0 to 1;

p = 1; R (branched or linear) = Man, oligomannose.



1. Endoglycanase

2. Galactosyltransferase, UDP-Gal

3. CMP-SA-PEG, ST3Gal3

a-m, p-y = 0; n (independently selected) = 0 or 1;

R' = -Gal-Sia-PEG.

FIG. 39I

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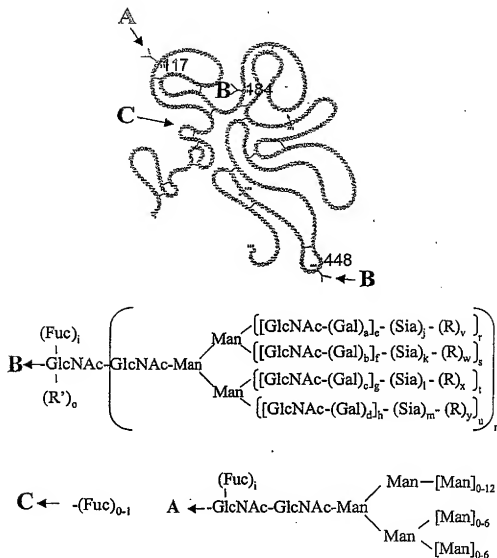
CHO, BHK, 293 cells, Vero expressed Cerezyme.  
a-d, i-m, q-u (independently selected) = 0 or 1;  
e-h = 1; v-y = 0.

- ↓
1. CMP-SA-linker-SA-CMP,  
ST3Gal3
  2. ST3Gal3, desialylated transferrin.
  3. CMP-SA, ST3Gal3

a-m, q-u (independently selected) = 0 or 1;  
p = 1; n = 0; v-y (independently selected) = 0 or 1;  
R = linker-transferrin.

FIG. 39J

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a-d, i, n-u (independently selected) = 0 or 1.

e-h (independently selected) = 0 to 4.

j-m (independently selected) = 0 to 20.

R = polymer; R' = sugar, glycoconjugate.

FIG. 40A



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CHO, BHK, 293 cells, Vero expressed tPA  
 a-g, n = 1; h = 1 to 3;  
 j-m, i, (independently selected) = 0 or 1;  
 r-u (independently selected) = 0 to 1; o, v-y = 0.

1. Mannosidase(s), sialidase
2. GNT1,2 (4 and/or 5) UDP-GlcNAc
3. Gal transferase, UDP-Gal
4. CMP-SA-PEG, ST3Gal3

A = B; a-g, n = 1; h = 1 to 3;  
 i, r-u (independently selected) = 0 or 1;  
 o = 0; j-m, v-y (independently selected) = 0 or 1;  
 R = PEG

FIG. 40B

Insect or fungi cell expressed tPA  
 A = B; a-d, f, h, j-o, s, u, v-y = 0;  
 e, g, i, n, r, t (independently selected) = 0 or 1.

1. GNT's 1&2, UDP-GlcNAc
2. Galactosyltransferase, UDP-Gal
3. CMP-SA-PEG, ST3Gal3

A = B; b, d, f, h, k, m, o, s, u, w, y = 0;  
 a, c, e, g, i, r, t (independently selected) = 0 or 1;  
 n = 1; j, l, v, x (independently selected) = 0 or 1;  
 R = PEG.

FIG. 40C

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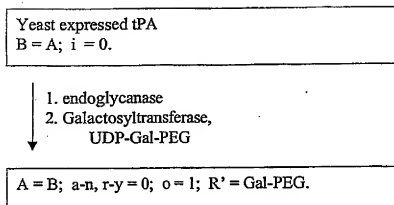


FIG. 40D

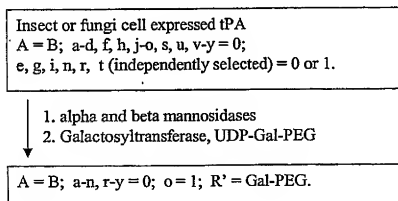


FIG. 40E

159/497

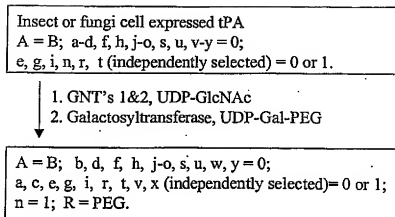


FIG. 40F

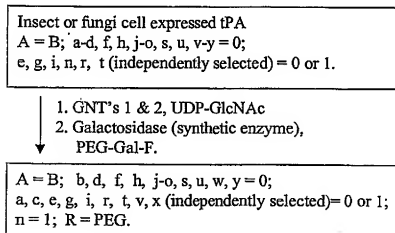
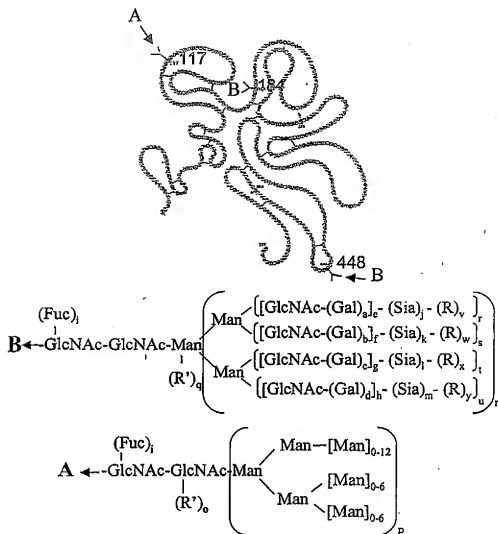


FIG. 40G

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a-d, i, n-u (independently selected) = 0 or 1.

e-h (independently selected) = 0 to 4.

j-m (independently selected) = 0 to 20.

R = polymer; R' = sugar, glycoconjugate.

FIG. 40H

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NSO expressed tPA

A = B; a-m, r-u (independently selected) = 0 or 1;  
n = 1; o, p, q, v-y = 0

- ↓
1. sialidase, alpha-galactosidase
  2. CMP-SA-levulinate, ST3Gal3,
  3. H<sub>4</sub>N<sub>2</sub>-PEG

A = B; a-m, r-y (independently selected) = 0 or 1;  
n = 1; o, p, q = 0;  
v-y (independently selected) = 1,  
when j-m (independently selected) is 1;  
R = PEG.

FIG. 40I

CHO, BHK, 293 cells, Vero expressed tPA

a-g, n, p = 1; h = 1 to 3;  
j-m, i, (independently selected) = 0 or 1;  
r-u (independently selected) = 0 to 1; q, o, v-y = 0.

- ↓
1. alpha and beta Mannosidases
  2. CMP-SA, ST3Gal3
  3. Galactosyltransferase, UDP-Gal-PEG

a-g, n = 1; h = 1 to 3;  
i, r-u (independently selected) = 0 or 1; o = 1;  
q, p, v-y = 0; j-m (independently selected) = 0 or 1;  
R' = Gal-PEG

FIG. 40J

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Plant expressed tPA

A = B; a-d, f, h, j-n, s, u, v-y = 0;

e, g, i, q, r, t (independently selected) = 0 or 1;

n = 1; R' = xylose

- ↓
1. hexosaminidase,
  2. alpha mannosidase and  
xylosidase
  3. GlcNAc transferase, UDP-  
GlcNAc-PEG

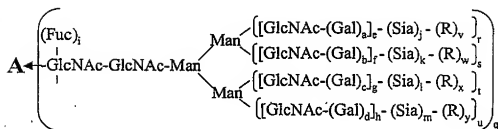
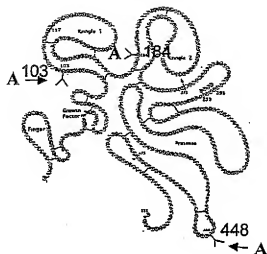
A = B; a-d, f, h, j-n, s, u, v-y = 0;

e, g, i, r, t (independently selected) = 0;

q = 1; R' = GlcNAc-PEG.

FIG. 40K

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a-d, i, q-u (independently selected) = 0 or 1.  
 e-h (independently selected) = 0 to 6.  
 j-m (independently selected) = 0 to 100.  
 v-y = 0; R = polymer.

FIG. 40L

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CHO, BHK, 293 cells, Vero expressed TNK tPA  
a-d, i-m, q-u (independently selected) = 0 or 1;  
e-h = 1; v-y = 0.

- ↓
1. Sialidase
  2. CMP-SA-PEG (16 mol eq),  
ST3Gal3

a-d, i-m, q-u (independently selected) = 0 or 1;  
e-h = 1; v-y (independently selected) = 1,  
when j-m (independently selected) is 1;  
R = PEG.

FIG. 40M

CHO, BHK, 293 cells, Vero expressed TNK tPA  
a-d, i-m, q-u (independently selected) = 0 or 1;  
e-h = 1; v-y = 0.

- ↓
1. Sialidase
  2. CMP-SA-PEG (1.2 mol eq),  
ST3Gal3
  3. CMP-SA (16 mol eq), ST3Gal3

a-d, i-m, q-u (independently selected) = 0 or 1;  
e-h = 1; v-y (independently selected) = 0 or 1;  
R = PEG.

FIG. 40N



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NSO expressed TNK tPA

a-d, i-m, q-u (independently selected) = 0 or 1;

e-h = 1; v-y = 0;

Sia (independently selected) = Sia or Gal.

- ↓
1. Sialidase and  $\alpha$ -galactosidase
  2. Galactosyltransferase, UDP-Gal
  - ▼ 3. CMP-SA-PEG, ST3Gal3

a-d, i-m, q-u (independently selected) = 0 or 1;

e-h = 1; v-y (independently selected) = 1,

when j-m (independently selected) is 1;

R = PEG.

FIG. 400



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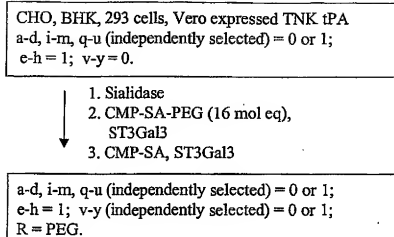


FIG. 40Q

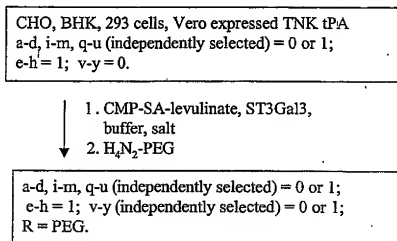


FIG. 40R

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CHO, BHK, 293 cells, Vero expressed TNK tPA  
a-d, i-m, q-u (independently selected) = 0 or 1;  
e-h = 1; v-y = 0.

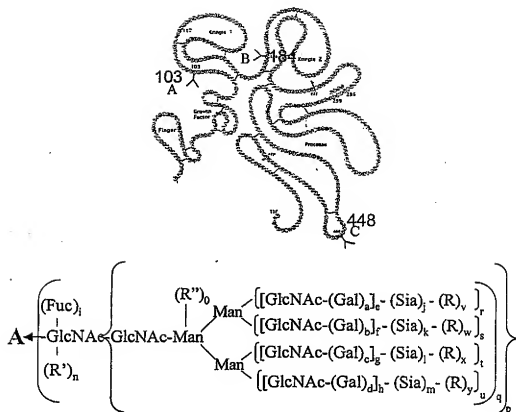


1. CMP-SA,  $\alpha$ 2,8-ST

a-d, i, q-u (independently selected) = 0 or 1;  
e-h = 1; j-m (independently selected) = 0-20;  
v-y (independently selected) = 0.

FIG. 40S

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a-d, i, n-y (independently selected) = 0 or 1.

e-h (independently selected) = 0 to 6.

j-m (independently selected) = 0 to 100.

R = modifying group, mannose, oligo-mannose;

R' = H, glycosyl residue, modifying group, glycoconjugate.

R'' = glycosyl residue.

FIG. 40T

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Insect cell expressed TNK tPA  
a-d, f, h, j-m, s, u, v-y = 0;  
e, g, i, q, r, t (independently selected) = 0 or 1.

- ↓
1. GNT's 1,2,4,5, UDP-GlcNAc
  2. Galactosyltransferase, UDP-Gal-PEG

a-i, q-u (independently selected) = 0 or 1;  
j-m = 0; v-y (independently selected) = 1,  
when e-h (independently selected) is 1;  
R = PEG.

FIG. 40U

Yeast expressed TNK tPA  
a-m = 0; q-y (independently selected) = 0 to 1; p = 1;  
R (branched or linear) = Man, oligomannose.

- ↓
1. Endoglycanase
  2. Galactosyltransferase, UDP-Gal-PEG

a-m, p-y = 0; n (independently selected) = 0 or 1;  
R' = -Gal-PEG.

FIG. 40V

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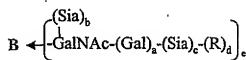
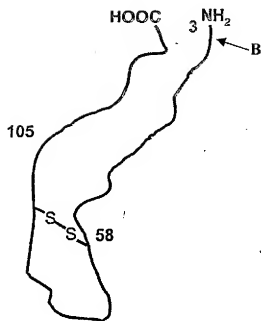
CHO, BHK, 293 cells, Vero expressed TNK tPA  
a-d, i-m, q-u (independently selected) = 0 or 1;  
e-h = 1; v-y = 0.

- ↓
1. CMP-SA-linker-Gal-UDP,  
ST3Gal3
  2. Galactosyltransferase, anti-TNF  
IG chimera produced in CHO.

a-m, r-u (independently selected) = 0 or 1; p, q = 1;  
n = 0; v-y (independently selected) = 0 or 1;  
R = linker-anti-TNF IG chimera protein.

FIG. 40W

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a-c, e (independently selected) = 0 or 1;  
 d = 0;  
 R = modifying group, mannose, oligo-  
 mannose.

FIG. 41A



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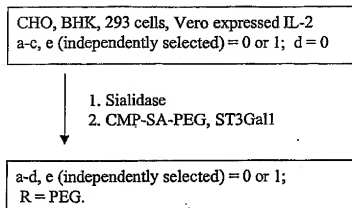


FIG. 41B

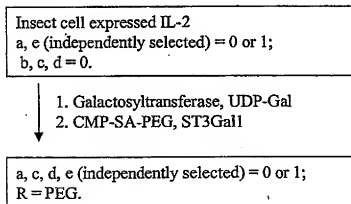


FIG. 41C

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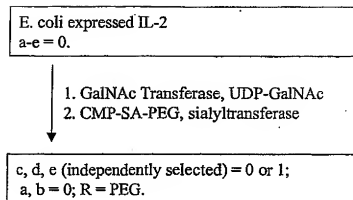


FIG. 41D

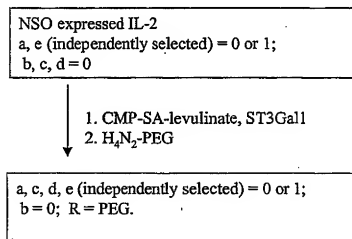


FIG. 41E

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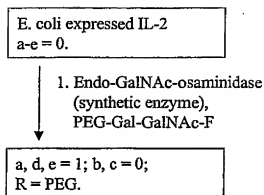


FIG. 41F

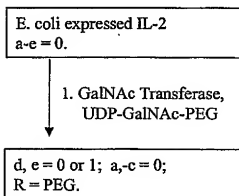
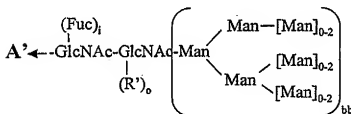
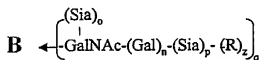
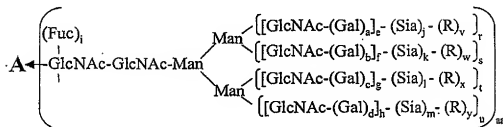


FIG. 41G

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2 peptides  
A and A' - N-linked sites  
B - O-linked sites



Alternate structure  
for some N-linked  
structures of A.

a-d, i, n-u (independently selected) = 0 or 1.  
aa, bb (independently selected) = 0 or 1.  
e-h (independently selected) = 0 to 6.  
j-m (independently selected) = 0 to 20.  
v-z = 0; R = polymer, glycoconjugate.

FIG. 42A

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CHO, BHK, 293s cells, Vero, MDCK, HEKC expressed  
Factor VIII.

e-h = 1 to 4;

aa, bb, a-d, j-m, i, n-u (independently selected) = 0 or 1;

v-z = 0.



1. Sialidase

2. CMP-SA-PEG, ST3Gal3

e-h = 1 to 4;

aa, bb, a-d, i, n, q-u (independently selected) = 0 or 1;

o, p, z = 0; j-m, v-y (independently selected) = 0 or 1;

R = PEG.

FIG. 42B

CHO, BHK, 293S cells, Vero, MDCK, 293S, HEKC  
expressed Factor VIII.

e-h = 1 to 4;

aa, bb, a-d, j-m, i, n-u (independently selected) = 0 or 1;

v-z = 0.



1. Sialidase

2. CMP-SA-PEG, ST3Gal3

3. ST3Gal1, CMP-SA

e-h = 1 to 4;

aa, bb, a-d, i, n, p-u (independently selected) = 0 or 1;

o, z = 0; j-m, v-y (independently selected) = 0 or 1;

R = PEG.

FIG. 42C

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CHO, BHK, 293s cells, Vero, MDCK, HEKC  
expressed Factor VIII.  
e-h = 1 to 4;  
aa, bb, a-d, j-m, i, n-u (independently selected)=0 or 1;  
v-z = 0.



1. CMP-SA-PEG, ST3Gal3

e-h = 1 to 4;  
aa, bb, a-d, i, n-u (independently selected) = 0 or 1;  
z = 0; j-m, v-y (independently selected) = 0 or 1;  
R = PEG.

FIG. 42D

CHO, BHK, 293S cells, Vero, MDCK, HEKC  
expressed Factor VIII.  
e-h = 1 to 4;  
aa, bb, a-d, j-m, i, n-u (independently selected) 0 or 1;  
v-z = 0.



1. CMP-SA-PEG, ST3Gal1

e-h = 1 to 4;  
aa, bb, a-d, i, n-u (independently selected) = 0 or 1;  
z = 0; j-m, v-y (independently selected) = 0 or 1;  
R = PEG.

FIG. 42E

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CHO, BHK, 293S cells, Vero, MDCK, HEKC  
expressed Factor VIII.

e-h = 1 to 4;

aa, bb, a-d, j-m, i, n-u (independently selected)=0 or 1;

v-z = 0.



1. CMP-SA-PEG,  $\alpha$ 2,8-ST

e-h = 1 to 4;

aa, bb, a-d, i, n-y (independently selected) = 0 or 1;

z = 0; j-m (independently selected) = 0 to 2;

v-y (independently selected) = 1,

when j-m (independently selected) is 2;

R = PEG.

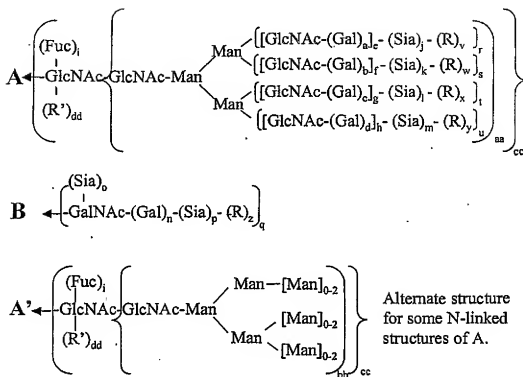
FIG. 42F

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2 peptides

A or A' - N-linked sites

B - O-linked sites



a-d, i, n-u, (independently selected) = 0 or 1.

aa, bb, cc, dd (independently selected) = 0 or 1.

e-h (independently selected) = 0 to 6.

j-m (independently selected) = 0 to 20.

v-z = 0;

R = modifying group, mannose, oligo-mannose.

R' = H, glycosyl residue, modifying group, .

glycoconjugate.

FIG. 42G



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CHO, BHK, 293S cells, Vero, MDCK, HEKC  
expressed Factor VIII.

e-h = 1 to 4;

aa, bb, cc, a-d, j-m, i, n-u (independently selected) = 0 or 1;  
dd, v-z = 0.

- ↓  
1. CMP-SA-levulinate, ST3Gal3,  
2.  $H_4N_2$ -PEG

e-h = 1 to 4;

aa, bb, cc, a-d, i, n-u (independently selected) = 0 or 1;  
dd, z = 0; j-m, v-y (independently selected) = 0 or 1;  
R = PEG.

FIG. 42H

CHO, BHK, 293S cells, Vero, MDCK, HEKC  
expressed Factor VIII.

e-h = 1 to 4;

aa, bb, cc, a-d, j-m, i, n-u (independently selected) = 0 or 1;  
dd, v-z = 0.

- ↓  
1. endo-H  
2. galactosyltransferase, UDP-Gal-PEG

e-h = 1 to 4;

aa, bb, dd, a-d, i, j-u (independently selected) = 0 or 1;  
cc, v-z = 0; R' = -Gal-PEG.

FIG. 42I

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CHO, BHK, 293S cells, Vero, MDCK, HEKC  
expressed Factor VIII.

e-h = 1 to 4;

aa, bb, cc, a-d, j-m, i, n-u (independently selected) = 0 or 1;

dd, v-z = 0.

- ↓
1. ST3Gal3, CMP-SA
  2. endo-H
  3. galactosyltransferase, UDP-Gal-PEG

e-h = 1 to 4;

aa, bb, dd, a-d, i, j-u (independently selected) = 0 or 1;

cc, v-z = 0; R' = -Gal-PEG.

FIG. 42J

CHO, BHK, 293S cells, Vero, MDCK, HEKC  
expressed Factor VIII.

e-h = 1 to 4;

aa, bb, cc, a-d, j-m, i, n-u (independently selected) = 0 or 1;

dd, v-z = 0.

- ↓
1. mannosidases
  2. GNT 1 & 2, UDP-GlcNAc
  3. galactosyltransferase, UDP-Gal-PEG

e-h = 1 to 4;

aa, a-d, i, j-y (independently selected) = 0 or 1;

bb, cc, dd, z = 0; R = PEG.

FIG. 42K

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CHO, BHK, 293S cells, Vero, MDCK, HEKC  
expressed Factor VIII.

e-h = 1 to 4;

aa, bb, cc, a-d, j-m, i, n-u (independently selected) = 0 or 1;  
dd, v-z = 0.

- ↓
1. mannosidases
  2. GNT-1, 2, 4 & 5; UDP-GlcNAc
  3. galactosyltransferase, UDP-Gal
  4. ST3Gal3, CMP-SA

e-h = 1 to 4;

aa, bb, cc, a-d, i, j-q (independently selected) = 0 or 1;  
dd, v-z = 0.

FIG. 42L

CHO, BHK, 293S cells, Vero, MDCK, HEKC  
expressed Factor VIII.

e-h = 1 to 4;

aa, bb, cc, a-d, j-m, i, n-u (independently selected) = 0 or 1;  
dd, v-z = 0.

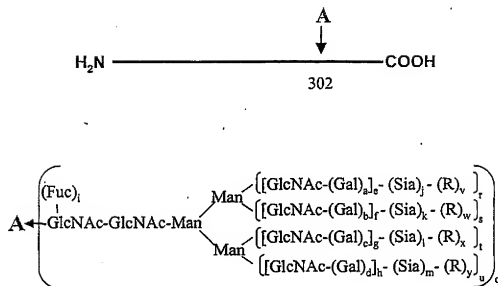
- ↓
1. mannosidases
  2. GNT-1, UDP-GlcNAc-PEG

e-h = 0 to 4;

aa, a-d, i, j-y (independently selected) = 0 or 1;  
bb, cc, dd, z = 0.

FIG. 42M

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a-d, i, q-u (independently selected) = 0 or 1.

e-h (independently selected) = 0 to 6.

j-m (independently selected) = 0 to 100.

v-y = 0; R = polymer.

FIG. 43A

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CHO, BHK, 293 cells, Vero expressed Urokinase.  
a-d, i-m, q-u (independently selected) = 0 or 1;  
e-h = 1; v-y = 0.



1. Sialidase
2. CMP-SA-PEG (16 mol eq),  
ST3Gal3

a-d, i-m, q-u (independently selected) = 0 or 1;  
e-h = 1; v-y (independently selected) = 1,  
when j-m (independently selected) is 1;  
R = PEG.

FIG. 43B

CHO, BHK, 293 cells, Vero expressed Urokinase.  
a-d, i-m, q-u (independently selected) = 0 or 1;  
e-h = 1; v-y = 0.



1. Sialidase
2. CMP-SA-PEG (1.2 mol eq),  
ST3Gal3
3. CMP-SA (16 mol eq), ST3Gal3

a-d, i-m, q-u (independently selected) = 0 or 1;  
e-h = 1; v-y (independently selected) = 0 or 1;  
R = PEG.

FIG. 43C

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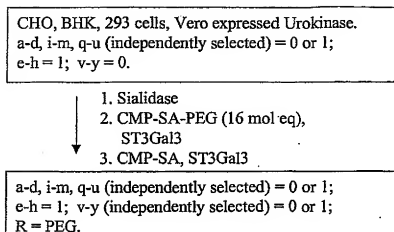


FIG. 43D

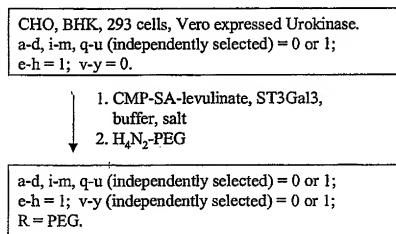


FIG. 43E

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CHO, BHK, 293 cells, Vero expressed Urokinase.  
a-d, i-m, q-u (independently selected) = 0 or 1;  
e-h = 1; v-y = 0.

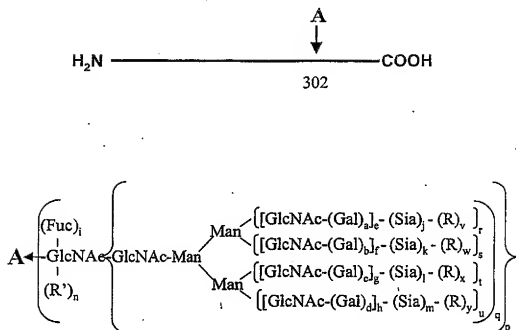


1. CMP-SA,  $\alpha$ 2,8-ST

a-d, i, q-u (independently selected) = 0 or 1;  
e-h = 1;  
j-m (independently selected) = 0-20;  
v-y (independently selected) = 0.

FIG. 43F

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a-d, i, n, p-u (independently selected) = 0 or 1.

e-h (independently selected) = 0 to 6.

j-m (independently selected) = 0 to 100.

v-y = 0;

R = modifying group, mannose, oligo-mannose;

R' = H, glycosyl residue, modifying group,  
glycoconjugate.

FIG. 43G



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Insect cell expressed Urokinase.

a-d, f, h, j-n, s, u, v-y = 0;

e, g, i, q, r, t (independently selected) = 0 or 1.



1. GNT's 1,2,4,5, UDP-GlcNAc

2. Galactosyltransferase, UDP-Gal-PEG

a-i, q-u (independently selected) = 0 or 1;

j-n = 0; v-y (independently selected) = 1,

when e-h (independently selected) is 1;

R = PEG.

FIG. 43H

Yeast expressed Urokinase.

a-n = 0;

q-y (independently selected) = 0 to 1;

p = 1; R (branched or linear) = Man, oligomannose.



1. Endoglycanase

2. Galactosyltransferase, UDP-Gal

3. CMP-SA-PEG, ST3Gal3

a-m, p-y = 0; n (independently selected) = 0 or 1;

R' = -Gal-Sia-PEG.

FIG. 43I

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CHO, BHK, 293 cells, Vero expressed Urokinase.  
 a-d, i-m, q-u (independently selected) = 0 or 1;  
 e-h = 1; n, v-y = 0.

1. CMP-SA-linker-SA-CMP, ST3Gal3
2. ST3Gal1, desialylated Urokinase produced in CHO.
3. CMP-SA, ST3Gal3, ST3Gal1

a-m, q-u (independently selected) = 0 or 1;  
 p = 1; n = 0;  
 v-y (independently selected) = 0 or 1;  
 R = linker-Urokinase.

FIG. 43J

Isolated Urokinase.  
 a-d, i-m, q-u (independently selected) = 0 or 1;  
 e-h = 1; v-y = 0; n = 0;  
 Sia (independently selected) = Sia or SO<sub>4</sub>;  
 Gal (independently selected) = Gal or GalNAc;  
 GlcNAc (independently selected) = GlcNAc or GlcNAc-Fuc.

1. sulfhydrylase
2. CMP-SA-PEG, sialyltransferase

a-d, i-m, q-u (independently selected) = 0 or 1;  
 n = 0; e-h = 1; Sia = Sia;  
 Gal (independently selected) = Gal or GalNAc;  
 GlcNAc (independently selected) = GlcNAc or GlcNAc-Fuc.  
 v-y (independently selected) = 0 or 1;  
 R = PEG.

FIG. 43K

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Isolated Urokinase.

a-d, i-m, q-u (independently selected) = 0 or 1;

e-h = 1; n = 0; v-y = 0;

Sia (independently selected) = Sia or SO<sub>4</sub>;

Gal (independently selected) = Gal or GalNAc;

GlcNAc (independently selected) = GlcNAc or GlcNAc-Fuc.



1. sulfohydrolase, hexosaminidase

2. UDP-Gal-PEG, galactosyltransferase

a-d, i, q-u (independently selected) = 0 or 1;

e-h = 1; j-n = 0; Gal (independently selected) = Gal;

GlcNAc (independently selected) = GlcNAc or GlcNAc-Fuc;

v-y (independently selected) = 0 or 1; R = PEG.

FIG. 43L



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CHO, BHK, 293 cells, Vero expressed DNase I.  
a-d, i-m, q-u (independently selected) = 0 or 1;  
e-h = 1; v-y = 0.

- ↓
1. Sialidase
  2. CMP-SA-PEG (16 mol eq),  
ST3Gal3

a-d, i-m, q-u (independently selected) = 0 or 1;  
e-h = 1;  
v-y (independently selected) = 1,  
when j-m (independently selected) is 1;  
R = PEG.

FIG. 44B

CHO, BHK, 293 cells, Vero expressed DNase I.  
a-d, i-m, q-u (independently selected) = 0 or 1;  
e-h = 1; v-y = 0.

- ↓
1. Sialidase
  2. CMP-SA-PEG (1.2 mol eq), ST3Gal3
  3. CMP-SA (16 mol eq), ST3Gal3

a-d, i-m, q-u (independently selected) = 0 or 1;  
e-h = 1; v-y (independently selected) = 0 or 1;  
R = PEG.

FIG. 44C

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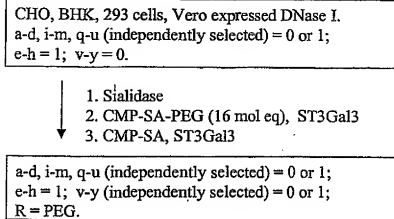


FIG. 44D

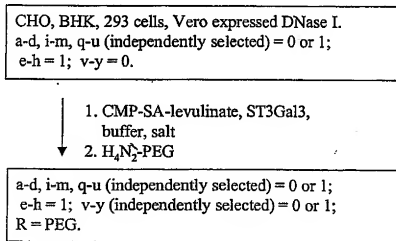


FIG. 44E

195/497

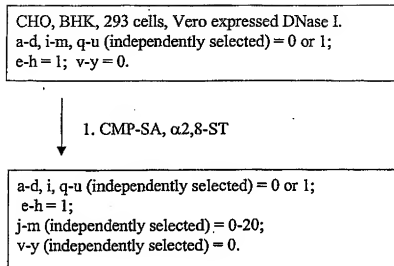
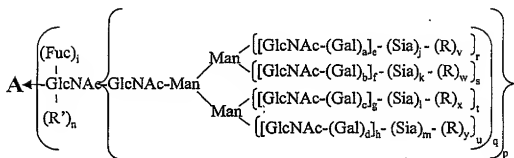
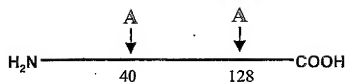


FIG. 44F

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a-d, i, n, p-u (independently selected) = 0 or 1.

e-h (independently selected) = 0 to 6.

j-m (independently selected) = 0 to 100.

v-y = 0;

R = modifying group, mannose, oligo-mannose;

R' = H, glycosyl residue, modifying group,  
glycoconjugate.

FIG. 44G



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Insect cell expressed DNase I.

a-d, f, h, j-n, s, u, v-y = 0;

e, g, i, q, r, t (independently selected) = 0 or 1.



1. GNT's 1,2,4,5, UDP-GlcNAc

2. Galactosyltransferase, UDP-Gal-PEG

a-i, q-u (independently selected) = 0 or 1; j-n = 0;

v-y (independently selected) = 1,

when e-h (independently selected) is 1;

R = PEG.

FIG. 44H

Yeast expressed DNase I.

a-n = 0;

q-y (independently selected) = 0 to 1;

p = 1; R (branched or linear) = Man, oligomannose.



1. Endoglycanase

2. Galactosyltransferase, UDP-Gal

3. CMP-SA-PEG, ST3Gal3

a-n, p-y = 0; n (independently selected) = 0 or 1;

R' = -Gal-Sia-PEG.

FIG. 44I

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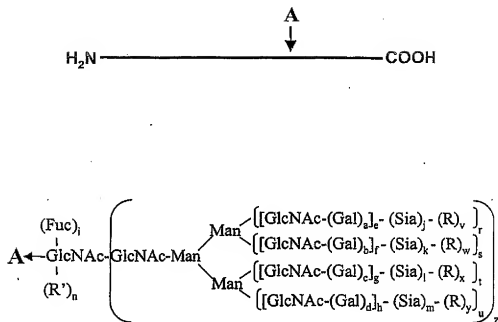
CHO, BHK, 293 cells, Vero expressed DNase I.  
a-d, i-m, q-u (independently selected) = 0 or 1;  
e-h = 1; n, v-y = 0.

- ↓
1. CMP-SA-linker-SA-CMP, ST3Gal3
  2. ST3Gal1, desialylated alpha-1-Proteinase inhibitor.
  3. CMP-SA, ST3Gal3, ST3Gal1

a-m, q-u (independently selected) = 0 or 1;  
p = 1; n = 0;  
v-y (independently selected) = 0 or 1;  
R = linker- alpha-1-Proteinase inhibitor.

FIG. 44J

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a-d, i, r-u (independently selected) = 0 or 1.

e-h (independently selected) = 0 to 4.

j-m (independently selected) = 0 or 1.

n, v-y = 0; z = 0 or 1;

R = modifying group, mannose, oligo-mannose;

R' = H, glycosyl residue, modifying group,  
glycoconjugate.

FIG. 45A

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CHO, BHK, 293 cells, Vero expressed Insulin.  
a-m, r-u (independently selected) = 0 or 1;  
n = 0; v-y = 0; z = 1.



1. Sialidase
2. CMP-SA-PEG, ST3Gal3

a-m, r-u (independently selected) = 0 or 1;  
v-y (independently selected) = 1,  
when j-m (independently selected) is 1;  
n = 0; R = PEG; z = 1.

FIG. 45B

Insect cell expressed Insulin.  
a-h, j-n, s-y = 0;  
i, r (independently selected) = 0 or 1; z = 1.



1. GNT's 1&2, UDP-GlcNAc-PEG

a-d, f, h, j-n, s, u, w, y = 0;  
e, g, i, r, t, v, x (independently selected) = 0 or 1;  
v, x (independently selected) = 1,  
when e, g (independently selected) is 1;  
z = 1; R = PEG.

FIG. 45C

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Yeast expressed Insulin.

a-n = 0; r-y (independently selected) = 0 to 1;

z = 1;

R (branched or linear) = Man, oligomannose or  
polysaccharide.



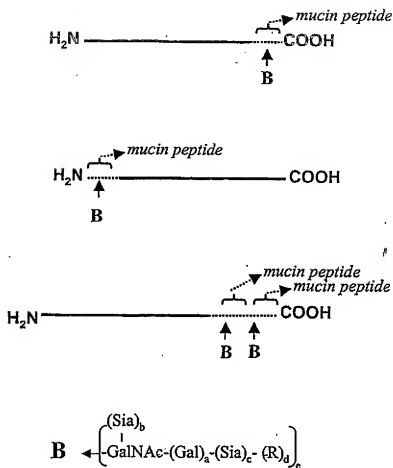
1. Endo-H

2. Galactosyltransferase, UDP-Gal-PEG

a-m, r-z = 0; n = 1; R' = -Gal-PEG.

FIG. 45D

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a-c, e (independently selected) = 0 or 1;  
 d = 0; R = polymer

FIG. 45E

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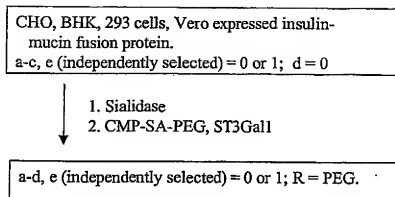


FIG. 45F

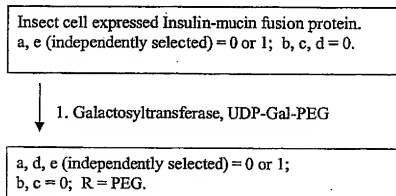


FIG. 45G

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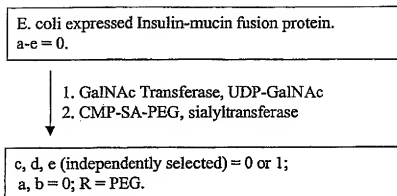
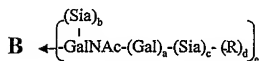
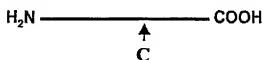
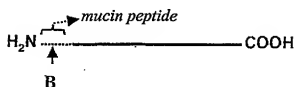


FIG. 45H



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a-c, e (independently selected) = 0 or 1;  
 d = 0; R = modifying group, mannose,  
 oligo-mannose.

FIG. 45I

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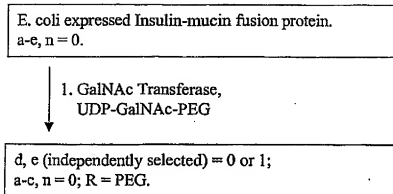


FIG. 45J

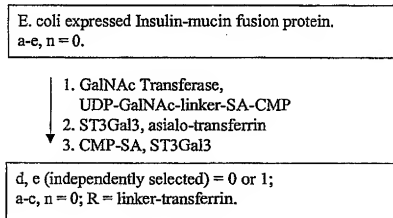


FIG. 45K

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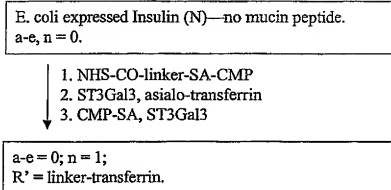
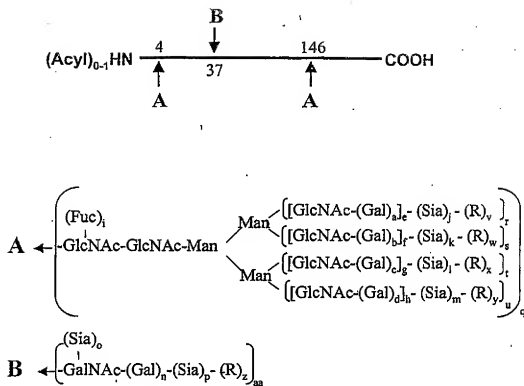


FIG. 45L

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a-d, i, n-u, aa (independently selected) = 0 or 1.

e-h (independently selected) = 0 to 6.

j-m (independently selected) = 0 to 100.

v-y = 0; R = polymer, glycoconjugate.

FIG. 46A

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CHO, BHK, 293 cells, Vero expressed M-antigen.  
 a-d, i-m, o-u, aa (independently selected) = 0 or 1;  
 n, e-h = 1; v-z = 0.

- ↓
1. Sialidase
  2. CMP-SA-linker-lipid-A,  
ST3Gal3

a-d, i-m, q-u, aa (independently selected) = 0 or 1;  
 o, p, z = 0; n, e-h = 1;  
 v-y (independently selected) = 1,  
 when j-m (independently selected) is 1;  
 R = linker-lipid-A.

FIG. 46B

CHO, BHK, 293 cells, Vero expressed M-antigen.  
 a-d, i-m, o-u, aa (independently selected) = 0 or 1;  
 n, e-h = 1; v-z = 0.

- ↓
1. sialidase
  2. CMP-SA-linker-tetanus toxin, ST3Gal1
  3. CMP-SA, ST3Gal3

a-d, i-m, p-u, z, aa (independently selected) = 0 or 1;  
 o, v-y = 0; n, e-h = 1; R = tetanus toxin.

FIG. 46C

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NSO expressed M-antigen.

a-d, i-n, o-u, aa (independently selected) = 0 or 1;

e-h = 1; v-z = 0;

Sia (independently selected) = Sia or Gal.

- ↓
1.  $\alpha$ -galactosidase
  2. CMP-SA, ST3Gal3
  2. CMP-SA-KLH, ST3Gal1

a-d, i-n, p-u, z, aa (independently selected) = 0 or 1;

e-h = 1; o, v-y = 0;

z = 1, when p = 1;

R = KLH.

FIG. 46D

Yeast expressed M-antigen.

a-p, z = 0; q-y, aa (independently selected) = 0 to 1;

R (branched or linear) = Man, oligomannose;

GalNAc = Man.

- ↓
1.  $\alpha$ 1,2-mannosidase
  2. GNT 1,
- ↓ UDP-GlcNAc-linker-diphtheria toxin.

e, q, l, m, r, t, u, v, aa (independently selected) = 0 or 1;

a-d, f-h, j, k, n-p, s, w-z = 0;

Sia = Man; R = linker-diphtheria toxin.

FIG. 46E

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CHO, BHK, 293 cells, Vero expressed M-antigen.  
a-d, i-m, o-u, aa (independently selected) = 0 or 1;  
n, e-h = 1; v-z = 0.



1. CMP-SA-levulinate, ST3Gal3,
2. H<sub>4</sub>N<sub>2</sub>-linker-DNA

a-d, i-m, o-y, aa (independently selected) = 0 or 1;  
z = 0; n, e-h = 1; R = linker-DNA.

FIG. 46F

CHO, BHK, 293 cells, Vero expressed M-antigen.  
a-d, i-n, o-u, aa (independently selected) = 0 or 1;  
e-h = 1; v-z = 0.

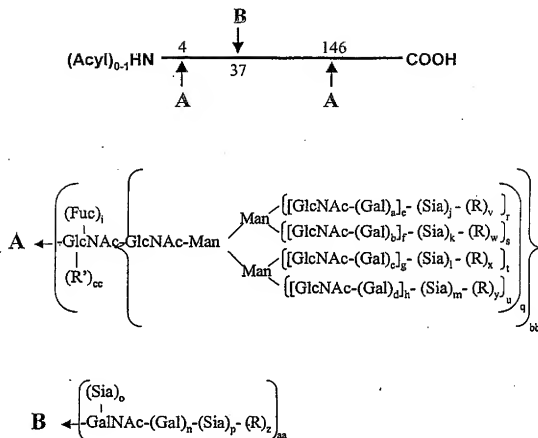


1. CMP-SA, poly- $\alpha$ 2,8-ST

a-d, i, n-u, aa (independently selected) = 0 or 1;  
e-h = 1; j-m (independently selected) = 0-100;  
v-z (independently selected) = 0.

FIG. 46G

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a-d, i, n, q-u, aa, bb, (independently selected) = 0 or 1.

e-h (independently selected) = 0 to 6.

j-p (independently selected) = 0 to 100.

Cc, v-y = 0;

R = modifying group, mannose, oligo-mannose.

R' = H, glycosyl residue, modifying group, glycoconjugate.

FIG. 46H



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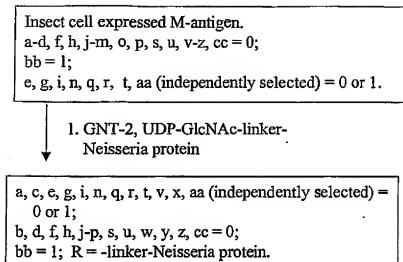


FIG. 46I

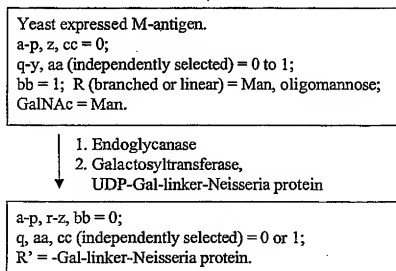


FIG. 46J

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Yeast expressed M-antigen.

a-p, z, cc = 0;

q-y, aa (independently selected) = 0 to 1; bb = 1;

R (branched or linear) = Man, oligomannose;

GalNAc = Man.

1. mannosidases

2. GNT 1 & 2, UDP-GlcNAc

3. UDP-Gal, Galactosyltransferase,

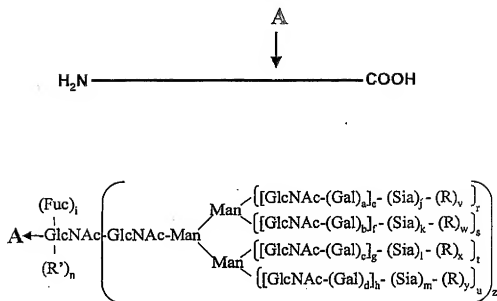
4. CMP-SA, sialyltransferase

a, c, e, g, j, l, q, r, t, aa (independently selected) = 0 or 1;

b, d, f, h, k, m-p, s, u-z, cc = 0; bb = 1.

FIG. 46K

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a-d, i, r-u (independently selected) = 0 or 1.

e-h (independently selected) = 0 to 4.

j-m (independently selected) = 0 or 1.

n, v-y = 0; z = 0 or 1;

R = modifying group, mannose, oligo-mannose;

R' = H, glycosyl residue, modifying group,  
glycoconjugate.

FIG. 47A

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CHO, BHK, 293 cells, Vero expressed Growth Hormone.

a-m, r-u (independently selected) = 0 or 1;  
n = 0; v-y = 0; z = 1.

- ↓
1. Sialidase
  2. CMP-SA-PEG, ST3Gal3

a-m, r-u (independently selected) = 0 or 1;  
v-y (independently selected) = 1,  
when j-m (independently selected) is 1;  
n = 0; R = PEG; z = 1.

FIG. 47B

Insect cell expressed growth hormone.

a-h, j-n, s-y = 0;  
i, r (independently selected) = 0 or 1; z = 1.

- ↓
1. GNT's 1&2, UDP-GlcNAc-PEG

a-d, f, h, j-n, s, u, w, y = 0;  
e, g, i, r, t, v, x (independently selected) = 0 or 1;  
v, x (independently selected) = 1,  
when e, g (independently selected) is 1;  
z = 1; R = PEG.

FIG. 47C

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Yeast expressed growth hormone.  
a-n = 0; r-y (independently selected) = 0 to 1;  
z = 1;  
R (branched or linear) = Man, oligomannose or  
polysaccharide.

- ↓  
1. Endo-H  
2. Galactosyltransferase, UDP-Gal-PEG

a-m, r-z = 0; n = 1; R' = -Gal-PEG.

FIG. 47D



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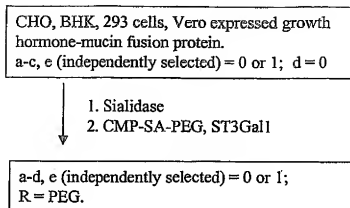


FIG. 47F

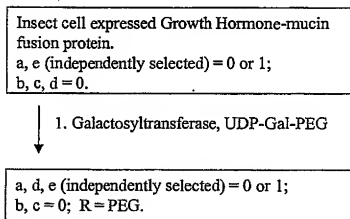


FIG. 47G

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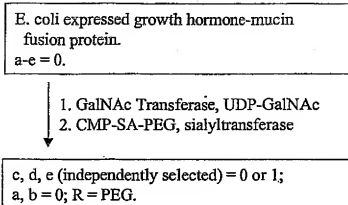


FIG. 47H

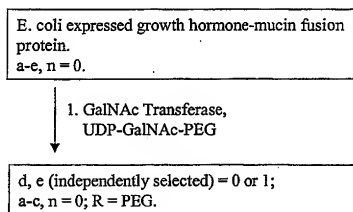


FIG. 47I



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E. coli expressed growth hormone-mucin fusion protein.

a-e, n = 0.

1. GalNAc Transferase,  
UDP-GalNAc-linker-SA-CMP
2. ST3Gal3, asialo-transferrin
3. CMP-SA, ST3Gal3

d, e (independently selected) = 0 or 1;  
a-c, n = 0; R = linker-transferrin.

FIG. 47J

E. coli expressed growth hormone  
(N)—no mucin peptide.

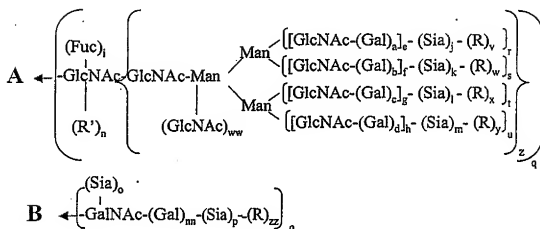
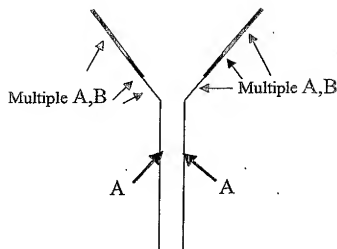
a-e, n = 0.

1. NHS-CO-linker-SA-CMP
2. ST3Gal3, asialo-transferrin
3. CMP-SA, ST3Gal3

a-e = 0; n = 1; R' = linker-transferrin.

FIG. 47K

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a-d, i-m, q-u, w, z, nn, ww, zz. (independently selected) = 0 or 1.

e-h (independently selected) = 0 to 4.

n, v-y = 0;

R = modifying group, mannose, oligo-mannose;

R' = H, glycosyl residue, modifying group, glycoconjugate.

FIG. 48A

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CHO, BHK, 293 cells, Vero or transgenic animals  
expressed TNF Receptor IgG Fusion.  
a-m, o-u, aa (independently selected) = 0 or 1;  
n = 1; v-z = 0.

- ↓
1. CMP-SA, ST3Gal1
  2. galactosyltransferase, UPD-Gal
  3. CMP-SA-PEG, ST3Gal3

a-m, o-u, v-y, aa (independently selected) = 0 or 1;  
n = 1; z = 0; R = PEG.

FIG. 48B

CHO, BHK, 293 cells, Vero expressed  
TNF Receptor IgG Fusion.  
a-m, o-u, aa (independently selected) = 0 or 1;  
n = 1; v-z = 0.

- ↓
1. sialidase
  2. CMP-SA-PEG, ST3Gal1

a-i, p-u, z, aa (independently selected) = 0 or 1;  
n = 1; o, j-m, v-y = 0; R = PEG.

FIG. 48C

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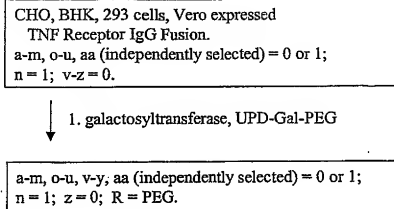


FIG. 48D

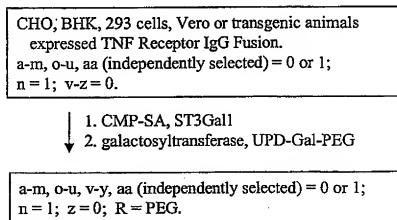


FIG. 48E

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CHO, BHK, 293 cells, Vero or transgenic animals  
expressed TNF Receptor IgG Fusion.

a-m, o-u, aa (independently selected) = 0 or 1;  
n = 1; v-z = 0.

↓  
1. CMP-SA-levulinate, ST3Gal1  
2. H<sub>4</sub>N<sub>2</sub>-PEG

a-m, o-u, v-y, aa (independently selected) = 0 or 1;  
n = 1; z = 0; R = PEG.

FIG. 48F

CHO, BHK, 293 cells, Vero expressed  
TNF Receptor IgG Fusion.

a-m, o-u, aa (independently selected) = 0 or 1;  
n = 1; v-z = 0.

↓  
1. CMP-SA-PEG,  $\alpha$ 2,8-ST

a-i, o, q-u, v-z, aa (independently selected) = 0 or 1;  
n = 1; j-m, p (independently selected) = 0 to 2;  
v-z (independently selected) = 1,  
when j-m, p (independently selected) is 2;  
R = PEG.

FIG. 48G